with claim 43 from which it depends. Support for amended claim 44 is found on page 19, lines 31-38; page 20, lines 33-38; and page 21, lines 4-11. Claim 60 has been amended for purposes of clarity. Support for amended claim 60 is found on page 9, lines 36-37; page 13, lines 28-29; page 23, lines 28-40; and page 24, lines 1-29. Claim 64 has been amended for purposes of clarity. Claims 43-45, 52-66, 108, 112 and 113 are pending. The amendments to the claims are indicated in the section entitled "Versions With Markings to Show Changes Made." A list of the now pending claims is provided in the section entitled "Pending Claims 43-45, 52-66, 108, 112 and 113, As Amended."

Claim Rejections - 35 U.S.C. §112, Second Paragraph

Claim 60 (and claims 61-63 which depend therefrom) stands rejected under 35 U.S.C. §112, second paragraph, as being indefinite for lacking antecedent basis.

Applicant has amended claim 60 to recite the composition of claims 43 or 113 wherein at least one of said single stranded targeting polynucleotides comprises at least one substituent. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the outstanding rejection.

Claim 44 stands rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant has amended claim 44 to recite the composition of claim 113 further comprising a secondary probe, wherein the probe is substantially complementary to at least one of the single stranded polynucleotides and wherein the probe forms a lock structure with at least one of the locking nucleic acids. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the outstanding rejection.

Claim 64 stands rejected under 35 U.S.C. §112, second paragraph, as being vague and indefinite. Applicant has amended claim 64 to recite a cell comprising a composition selected from claim 43. Amended claim 64 no longer depends, in part, from claims which have been cancelled. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the outstanding rejection.

Claim Rejections - 35 U.S.C. §102(e) As Being Anticipated by *Pati, et al.* (U.S. Patent 5,948,653)

Claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 stand rejected under 35 U.S.C. §102(e) as being anticipated by *Pati et al.* (U.S. Patent 5,948,653).

Pati, et al. teaches formation of a four-stranded double D loop comprising a recombinase and at least two single-stranded targeting polynucleotides which are substantially complementary to each other wherein the targeting polynucleotides each comprise at least one homology clamp substantially corresponding to or substantially complementary to a preselected target nucleotide sequence (*see e.g.,* column 19, lines 22-28 and column 22, lines 14-15). The double D loop taught in *Pati* is stabilized by Watson-Crick base-pairing in that each single-stranded targeting polynucleotide disclosed in the reference base pairs via Watson-Crick interactions with one of the displaced strands of target duplex DNA (*see e.g.,* column 22, lines 4-7 and 17-24 and Fig. 13).

In contrast to *Pati*, Applicant's amended claims 43, 113, 108, and 112 require a locking nucleic acid that is positioned between a first and second homology clamp in which the locking nucleic acid stabilizes the complex so formed by a secondary structure. Applicant is unaware of any disclosure in *Pati* which teaches or suggests locking nucleic

acids which confer stable secondary structure to complexes comprising first and second homology clamps.

Accordingly, *Pati* does not teach all of the limitations of the amended claims. Applicant therefore requests that the outstanding rejection under 35 U.S.C. §102(e) of claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 be withdrawn.

Claim Rejections - 35 U.S.C. §103(a) As Being Unpatentable under *Pati, et al.* in view of Secondary References *Helene, et al., Barton, et al.* (U.S. Patent 5,225,556), or *Simonsson, et al.*

Claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 stand rejected under 35 U.S.C. §103(a) as being unpatentable under *Pati et al.* (U.S. Patent 5,948,653) in view of *Helene, et al.*

Claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 stand rejected under 35 U.S.C. §103(a) as unpatentable under *Pati et al.* (U.S. Patent 5,948,653) in view of *Barton, et al.* (U.S. Patent 5,225,556).

Claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 stand rejected under 35 U.S.C. §103(a) as being unpatentable under *Pati et al.* (U.S. Patent 5,948,653) in view of *Simonsson, et al.*

Pati et al, as discussed herein, does not teach or suggest locking nucleic acids which confer stable secondary structure to complexes comprising first and second homology clamps.

Helene, et al. teaches the mechanisms by which synthetic oligonucleotides and antisense RNA may interfere with gene expression. The reference discloses that triple helices may be formed between probe nucleic acids and double-stranded target DNA (*see*

e.g., page 102 and Fig. 3). As distinguished from *Helene*, Applicant's amended claims require a locking nucleic acid that is positioned between a first and second homology clamp in which the locking nucleic acid stabilizes the complex so formed by a secondary structure. *Helene* neither teaches nor suggests double-D loops, homology clamps, or locking nucleic acids which confer secondary structure to complexes comprising homology clamps. *Pati*, similarly, neither teaches nor suggests locking nucleic acids which confer stable secondary structure to complexes comprising homology clamps. Accordingly, the combined teachings of *Helene* and *Pati* do not teach or suggest each and every limitation of the amended claims. Applicant therefore requests that the outstanding rejection under 35 U.S.C. §103(a) of claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 be withdrawn.

Barton, et al. teaches complexes useful for the labeling, nicking and cleaving of Z-DNA and A-DNA (*see e.g.*, abstract) using 1,10 phenanthroline (and substituted derivatives thereof) (*see e.g.*, column 4, lines 12-68 and column 5, lines 1-12) and 3,4,7,8-tetramethyl-phenanthroline (and substituted derivatives thereof) (*see e.g.*, column 5, lines 13-68 and column 6, lines 1-30). *Barton, et al.*, like *Helene*, neither teaches nor suggests double-D loops, homology clamps, or locking nucleic acids which confer secondary structure to complexes comprising homology clamps. *Pati*, similarly, neither teaches nor suggests locking nucleic acids which confer stable secondary structure to complexes comprising homology clamps. Accordingly, the combined teachings of *Barton* and *Pati* do not teach or suggest each and every limitation of the amended claims. Applicant therefore requests that the outstanding rejection under 35 U.S.C. §103(a) of claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 be withdrawn.

Simonsson, et al. teaches that the major control element of the human *c-myc* oncogene forms a tetraplex which may play a role in oncogene activation (*see e.g.*,

abstract and page 1167). Like *Helene* and *Barton*, *Simonsson* fails to teach or suggest double-D loops, homology clamps, or locking nucleic acids which confer secondary structure to complexes comprising homology clamps. *Pati*, similarly, neither teaches nor suggests locking nucleic acids which confer secondary structure to complexes comprising homology clamps. Accordingly, the combined teachings of *Simonsson* and *Pati* do not teach or suggest each and every limitation of the amended claims. Applicant therefore requests that the outstanding rejection under 35 U.S.C. §103(a) of claims 43 (and claims 45, 52-66 and 113 which depend therefrom), 108 and 112 be withdrawn.

Claim Rejections - 35 U.S.C. §102(b) As Being Anticipated by *Sena, et al.* (U.S. Patent 5,273,881).

Claims 43 (and claims 52, 54-56, 60-63, and 113 which depend therefrom) and 108 stand rejected under 35 U.S.C. §102(b) as being anticipated by *Sena et al.* (U.S. Patent 5,273,881).

Sena, et al. teaches a set of two DNA probes each containing sequences complementary to a first target sequence strand or to a second target sequence strand wherein the probes additionally contain a region of complementary overlap. The probes in *Sena* are coated with recombinase and combined with a linear duplex DNA which contains a target sequence under conditions which produce a probe:target complex comprising both probes strands and both strands of linear duplex DNA (*see e.g.*, column 3, lines 44-55).

In contrast to *Sena,* the amended claims require a locking nucleic acid that is positioned between a first and second homology clamp in which the locking nucleic acid sequence stabilizes the complex so formed by a secondary structure. Applicant is unaware of any disclosure in *Sena* which teaches or suggests locking nucleic acids which confer

secondary structure to complexes comprising first and second homology clamps. Accordingly, *Sena* does not teach all of the limitations of the amended claims. Applicant therefore requests that the outstanding rejection under 35 U.S.C. §102(b) of claims 43 (and claims 52, 54-56, 60-63, and 113 which depend therefrom) and 108 be withdrawn.

Conclusion

If upon, review, the Examiner feels there are additional outstanding issues, the Examiner is invited to direct any calls in connection with this application to the undersigned at (415) 781-1989.

Respectfully submitted,
DORSEY & WHITNEY LLP

Date 12-16-02

Nancy Copps
Reg. No. 45,638 for
Richard F. Trecartin, Reg. No. 31,801

Four Embarcadero Center, Suite 3400
San Francisco, California 94111-4187
Telephone: (415) 781-1989

1093790

VERSIONS WITH MARKINGS TO SHOW CHANGES MADE

43. (Amended) A composition comprising at least one recombinase and a double D-loop comprising a target nucleic acid and a first single stranded targeting polynucleotide comprising a first homology clamp that is substantially complementary to a preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and [at least one locking sequence] a first locking nucleic acid positioned between said first and second homology clamps of said first polynucleotide wherein said first locking nucleic acid stabilizes the complex so formed by a secondary structure.

113. (Amended) The composition of claim 43 further comprising a second single stranded targeting polynucleotide comprising a first homology clamp that is substantially complementary to said preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and [at least one locking sequence] a second locking nucleic acid positioned between said first and second homology clamps of said second polynucleotide wherein said second locking nucleic acid stabilizes the complex so formed by a secondary structure.

44. (Amended) The composition of claim 113 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said single stranded polynucleotides and wherein said probe forms a lock structure with at least one of said locking [sequence] nucleic acids.

60. (Amended) The composition of claims 43 or 113 wherein at least one of said single stranded targeting [single stranded nucleic acids] polynucleotides comprises at least one substituent.

64. (Amended) A cell comprising a composition selected from claim[s 1, 20, or] 43.

108. (Amended) A kit comprising at least one recombinase and [two] a first and a second [substantially complementary] single stranded targeting polynucleotide[s], wherein said first and said second targeting polynucleotides are substantially complementary to each other and further wherein said first and said second targeting polynucleotides each [comprising] comprise:

a) [at least one] a first and a second homology clamp [that] wherein said first and said second homology clamps substantially correspond[s] to or [is] are substantially complementary to a preselected target nucleic acid sequence; and

b) [at least one locking sequence] a locking nucleic acid positioned between said first and said second homology clamps wherein said locking nucleic acid stabilizes the complex so formed by a secondary structure.

112. (Amended) A composition comprising a double D-loop comprising a target nucleic acid and [two] a first and a second [substantially complementary] single stranded targeting polynucleotide[s], wherein said first and said second targeting polynucleotides are substantially complementary to each other and further wherein said first and said second targeting polynucleotides each [comprising.] comprise:

i) [at least one] a first and second homology clamp [that] wherein said first and said second homology clamps substantially correspond[s] to or [is] are substantially complementary to a preselected target nucleic acid sequence of said target nucleic acid and to each other; and

ii) [at least one locking sequence] a locking nucleic acid positioned between said first and said second homology clamps wherein said locking nucleic acid stabilizes the complex so formed by a secondary structure and

[;] wherein said locking nucleic acid [sequence] forms a lock and further wherein a protein binds to said lock.

Pending Claims 43-45, 52-66, 108, 112 and 113, As Amended

43. (Amended) A composition comprising at least one recombinase and a double D-loop comprising a target nucleic acid and a first single stranded targeting polynucleotide comprising a first homology clamp that is substantially complementary to a preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and a first locking nucleic acid positioned between said first and second homology clamps of said first polynucleotide wherein said first locking nucleic acid stabilizes the complex so formed by a secondary structure.

113. (Amended) The composition of claim 43 further comprising a second single stranded targeting polynucleotide comprising a first homology clamp that is substantially complementary to said preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and a second locking nucleic acid positioned between said first and second homology clamps of said second polynucleotide wherein said second locking nucleic acid stabilizes the complex so formed by a secondary structure.

44. (Amended) The composition of claim 113 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said single stranded polynucleotides and wherein said probe forms a lock structure with at least one of said locking nucleic acids.

45. The composition of claim 113 wherein said locking sequences form an anchor structure from the group consisting of a triplex anchor, a quadruplex anchor, a Z-DNA anchor, and an A-DNA anchor.

52. The composition of claim 113 wherein at least one of said targeting polynucleotides comprises a peptide nucleic acid.

53. The composition of claim 113 wherein said locking sequences comprise DNA and RNA.

54. The composition of claim 113, wherein said recombinase is a species of prokaryotic recombinase.

55. The composition of Claim 54, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.

56. The composition of Claim 55, wherein said RecA protein species is *E. coli* RecA.

57. The composition of claim 43, wherein said recombinase is a species of eukaryotic recombinase.

58. The composition of claim 57, wherein said recombinase is a Rad51 recombinase.

59. The composition of claim 57, wherein said eukaryotic recombinase is a complex of recombinase proteins.

60. (Amended) The composition of claims 43 or 113 wherein at least one of said single stranded targeting polynucleotides comprises at least one substituent.

61. The composition of claim 60 wherein said substituent is a chemical substituent.

62. The composition of claim 60 wherein said substituent is a protein.

63. The composition of claim 60 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification tag moieties, and nucleic acid modification moieties.

64. (Amended) A cell comprising a composition selected from claim 43.

65. The cell of claim 64 which is a eukaryotic cell.

66. The cell of claim 64 which is a prokaryotic cell.

108. (Amended) A kit comprising at least one recombinase and a first and a second single stranded targeting polynucleotide, wherein said first and said second targeting polynucleotides are substantially complementary to each other and further wherein said first and said second targeting polynucleotides each comprise:

a) a first and a second homology clamp [that] wherein said first and said second homology clamps substantially correspond to or are substantially complementary to a preselected target nucleic acid sequence; and

b) a locking nucleic acid positioned between said first and said second homology clamps wherein said locking nucleic acid stabilizes the complex so formed by a secondary structure.

112. (Amended) A composition comprising a double D-loop comprising a target nucleic acid and a first and a second single stranded targeting polynucleotide, wherein said first and said second targeting polynucleotides are substantially complementary to each other and further wherein said first and said second targeting polynucleotides each comprise:

i) a first and second homology clamp wherein said first and said second homology clamps substantially correspond to or are substantially complementary to a preselected target nucleic acid sequence of said target nucleic acid and to each other; and

ii) a locking nucleic acid positioned between said first and said second homology clamps wherein said locking nucleic acid stabilizes the complex so formed by a secondary structure and wherein said locking nucleic acid forms a lock and further wherein a protein binds to said lock.